IN VITRO EFFECTS OF THE SELECTED BROMINATED FLAME RETARDANTS ON THE OVARIAN FOLLICULAR CELL STEROIDOGENESIS.

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Introduction

Polybrominated diphenyl ethers (PBDEs) are used in large quantities as additive flame retardants in plastics and textile materials. PBDEs can accumulate in the environment and have been detected in wildlife and in human adipose tissue and plasma samples. The congeners 2,2´,4,4´-tetraBDE (BDE-47), 2,2´,4,4´,5-pentaBDE (BDE-99), and 2,2´,4,4´,6-pentaBDE (BDE-100) are generally the dominant congeners found in wildlife and humans. BDE209, the main congener (> 97%) in the Deca-BDE formulation, was detected only in a minority of the samples and always at concentrations around the limit of detection.

Surprisingly there is a lack of data showing a direct action of brominated biphenyls on ovarian function and especially on the ovarian steroidogenesis.

In our laboratory, we collect follicular cells from porcine ovary excised from normal, hormone-untreated animals, to study the direct effects of xenobiotics on ovarian steroidogenesis. Pigs exhibit a wide range of physiological similarities to humans, notably, in metabolism, making porcine cells a valuable tool for studying potential human toxicants. We use a co-culture model of theca and granulosa cells cultured at the ratio existing in vivo in the follicles. This model is more physiological than the culture of individual cell types or cell lines. In this co-culture model, both cell types retain their developmental and follicular stage-specific ability to secrete steroids (estradiol, testosterone and progesterone). This model is, therefore, ideally suited for investigation of the effects of xenobiotics on hormone production by ovarian follicles. Surprisingly, data concerning direct action of a BDEs on ovarian function are scare.

The purpose of this research was to 1) determine estrogenic activity of BDE-47, BDE-99, BDE-100, BDE-209 in ovarian follicular cells , 2) determine their action on cell proliferation, viability and apoptosis.

Materials and Methods

Chemicals

In this study, cells were exposed to the polybrominated diphenyl ethers (PBDEs) numbers 47, 99, 100, and 209 (LGC Promochem). These congeners were chosen based on the concentration of BDE in the natural mixture extracted from liver oil from Burbot Mjosa and added at the ratio existing in the natural mixture:1000ng/ml BDE-47, 500 ng/ml BDE-99, 250 ng/ml BDE-100 and 10 ng/ml BDE-209).

Cell cultures

Granulosa cells (Gc) and theca interna cells (Tc) were isolated from antral prepubertal porcine ovarian follicles (4-6 mm in diameter) according to the technique described by Stokłosowa et at., (1978). For co-culture experiments, granulosa and theca cells were inoculated at concentrations of 4.0

x 10^4 and 1.0 x 10^4 viable cells/well, respectively, in 96-well tissue culture plates. This ratio is similar to that observed in vivo (Gc: Tc = 4:1) according to Stokłosowa et al., (1982). Cells were cultured in M199/FBS for 24hrs to allow for cell attachment. Then the cells were cultured for additional 48 h with particular congeners of BDEs.

Media were collected and used for cell viability determination (LDH kit, Roche Applied Science, Germany) and steroid analysis (testosterone and estradiol; Enzyme Immonoassay Kit (IBL, Hamburg, Germany). Cells lysates were incubated with caspase-3 colorimetric substrate (Sigma) or used for protein concentration measurement (Bradford). Every treatment was conducted in quadruplets (4 wells each), and each experiment was repeated 3 times.

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Results and Discussion

Steroid secretion under the influence of different BDEs present at the highest amount in natural mixture

All investigated congeners increased testosterone secretion (646.8 %; 615.6 %; 832.5 % and 1515 % of control in BDE-47-, BDE-99-, BDE-100- and BDE-209-treated cells, respectively).

All investigated congeners except for BDE-209 increased estradiol secretion (227.23 %; 266.2 %; 181.3 % and 160.9 % of control in BDE-47-, BDE-99-, BDE-100- and BDE-209-treated cells, respectively).

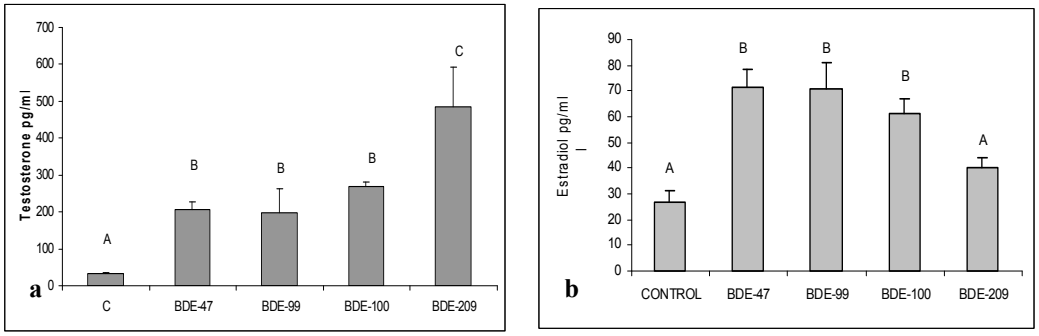

Fig.1 Testosterone (a) and estradiol (b) secretion in cells treated with different BDE congeners. All means marked with different letters are significantly different ($p < 0.05$) from the respective to control.

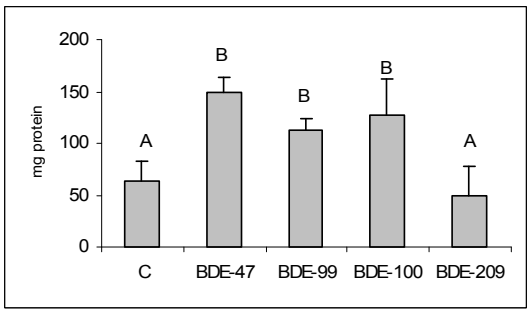

Fig. 2 Protein content in cultures treated with different BDE congeners. All means marked with different letters are significantly different ($p < 0.05$) from the respective to control.

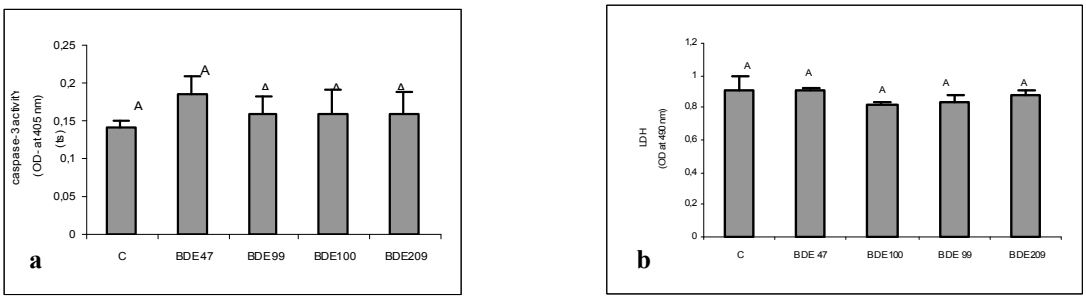

Fig. 3 Caspase-3 activity (a) and cell viability (b) in cells treated with different BDE congeners. All means marked with different letters are significantly different ($p < 0.05$) from the respective to control.

Results and discussion

There is little information available on the reproductive effects of BFRs. Studies with a commercial mixture of penta-BDE found a delay in male and female reproductive development at its high doses (Birnbaum & Staskal 2004). Recent reports demonstrated that a one-time, low-dose penta-BDE (BDE-99) exposure in utero resulted in the decreased sperm counts (Kuriyama et al. 2005).

Results of the present study indicate that all investigated congeners except for BDE-209 can act in ovarian follicles as estrogen receptor agonists with the following order of potency: BDE-47 =BDE-99> BDE-100. Indeed, some studies have shown that BFRs are estrogenically active (Fowles et al. 1994, Meerts et al. 2001, Zhou et al. 2001), and that this activity is similar to the behavior of bisphenol A, a well-known environmental estrogen (Meerts et al. 2001).

The increase in estradiol secretion induced by BDE-47, 99 and 100 was parallel to the increase in testosterone secretion, suggesting their action on CYP 17 activity. Admittedly, Stoker et al. (2005) recently showed that the PBDE mixture, DE-71, delayed puberty and suppressed the growth of androgen-dependent tissues in male Wistar rat and suggested that DE-71 might be either inducing steroid hormone metabolism or acting as an androgen receptor (AR) antagonist. However, PBDE 99 was found to be among the congeners that did not inhibit dihydrotestosterone-induced transcriptional activation at androgen receptors in MDA-kb2 cells (Stoker et al.2005).

Activation of CYP17 and activation or inhibition of CYP 19 activity have been proposed based on experiments with H295R cell line (Canton et al. 2005, 2006). Canton et al. (2006) in his paper showed that BDE99 and BDE100 only at the highest concentration significantly inhibited CYP17 activity in H295R Human Adrenocortical Carcinoma Cells. A strong inhibition of CYP17 was noted under the influence of a BDE47 metabolite (6OH-BDE47). In the latter study, the same regroup showed inhibitory and inductive effects on aromatase (CYP19) (the key enzyme that converts androgens to estrogens) induced by certain BFRs, in particular the hydroxylated PBDEs and several bromophenols (Canton et al. 2005).

Estrogenic action of these compounds rescheduled cell proliferation but had no effect on cell viability and apoptosis. So it is possible that higher steroid secretion is the consequence of the

increased proliferation. However, the T: E2 ratios in BDE-209- BDE -100-, BDE-99- and BDE- 47-exposed cells were 12:1; 6:1; 3;1 and 3:1, respectively, compared with 1.3: 1 in control culture. These results point to a possible inhibitory action of BDE-209 and stimulatory action of other investigated congeners on aromatase activity.
Since the presented results are the first demonstration of direct action of BDEs in the ovary, further studies are necessary to explain their mechanisms of action.

Acknowledgements

This work was supported partly by the Project 'Endocrine disrupters (ED): Risk assessment for food quality and animal heath", NFR:158849/I10, financed by the Norwegian Research Council 2004-2008. We thank Odd Egil Vestgarden at Peter Möller, Oslo. This study was also supported party by DS./BiNOZ/IZ/772, Poland.

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